Dipeptidyl peptidase IV Inhibitors

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The patent literature for dipeptidyl peptidase IV (DPP-IV) inhibitors for the period of January 2001 to May 2002 is reviewed. There has been increased interest in DPP-IV inhibitors since their potential for the treatment of diabetes was identified. This review will focus on reversible inhibitors of the enzyme, for which the primary interest has been for use in the treatment of Type II diabetes. The majority of the new chemical entities reported are dipeptide-like inhibitors that mimic the preferred substrates and the best of these display nanomolar activity. There have been fewer reports of non-peptide inhibitors suggesting that it is much more difficult to identify new classes of inhibitors. In addition to new chemical entities this review will cover new indications for DPP-IV inhibitors that have been identified using previously reported inhibitors as research tools.

Introduction

Dipeptidyl peptidase IV (DPP-IV, also known as CD26 or EC 3.4.14.5) is a membrane-bound serine protease first reported in 1966 that exhibits no sequence similarity with the classical serine protease families, but belongs to the prolyl oligopeptidase family [1••]. It is a highly specific aminopeptidase with unusual enzyme activity. It cleaves Xaa-Pro dipeptides from the N-terminus of peptides and proteins and Xaa-Ala dipeptides are also cleaved to a lesser extent. Other proline-specific proteases have been reported recently [2], some of which are not structurally homologous to DPP-IV but have similar enzyme activity. These include the closely related DPP8 and DPP9 claimed by the University of Sydney in the Patent Cooperation Treaty (PCT) application WO-00119866 [101] and Ferring BV in WO-00231134 [102]. There is a high sequence homology between the human, rat and mouse DPP-IV enzymes [3].

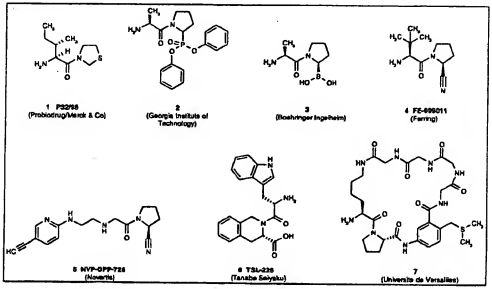
The enzyme is remarkably stable with a half-life of more than 48 h and is widely distributed in mammalian tissues, being found in the kidney, liver, intestinal epithelium, placenta, prostate, pancreas and blood plasma [4•,5]. There are also reports of the truncated soluble form being found in body fluids such as human serum, saliva, urine and synovial fluid. DPP-IV has been implicated in the regulation of the immune system [6-8] and is a well-established marker of T-lymphocyte activation. It has also been implicated in the regulation of inflammatory, nervous and endocrine functions [9].

The unique substrate specificity of DPP-IV results in the enzyme playing a key role in the metabolism of neuropeptides, immunopeptides and peptide hormones containing Xaa-Pro or Xaa-Ala amino terminal sequences [10-12]. Biologically active peptides with proven DPP-IV susceptibility include substance P, neuropeptide Y, peptide YY, enterostatin, growth-hormone-releasing factor (GRF) and the incretin hormones including glucose-dependent insulinotropic polypeptide (GIP) and the glucagon-like peptides.

Recently the emphasis of the research related to DPP-IV has been concentrated on its ability to cleave a His-Ala dipeptide from the N-terminus of the 30-amino acid peptide hormone, glucagon-like peptide (GLP-1 (7-36)). This hormone is regarded as the most important incretin. It stimulates glucose-dependent insulin secretion, inhibits hepatic glucose production and lowers blood glucose. In addition to its insulinotropic effects GLP-1 has been shown to promote the growth and differentiation of β-cells. GLP-1 is responsible for a substantial part of the insulin response to oral glucose and its function is greatly impaired in Type II diabetes. Since GLP-1 appears to play such an important role in management of glucose levels, GLP-1-based therapies are of potential use in treatment of non-insulin-dependent diabetes mellitus (Type II diabetes) [13,14]. Normalized glucose levels are achieved if circulating levels of GLP-1 are increased by 3- to 4-fold. However, the use of GLP-1 is limited to continuous intravenous infusion due to its short *in vivo* half-life. Treatment with DPP-IV inhibitors prevents the degradation of endogenous GLP-1 and the resulting increase in GLP-1 levels leads to enhanced insulin secretion and improved glucose tolerance in both normal and diabetic rats [15••,16,17]. The use of DPP-IV inhibitors has been proposed as a possible treatment of Type II diabetes.

The substrate requirements of the enzyme have been investigated [18,19] and an N-terminal primary or secondary amine at the P_1 position is an essential requirement. Data from these studies were utilized in the design of the first DPP-IV inhibitors. Although several classes of DPP-IV inhibitors have been reported [20••], by far the most successful have been dipeptide analogs of the natural substrates, one of the simplest of these being Ile-thiazolidide (1, P32/98; Figure 1), developed by Probiodrug AG [21] and licensed by Merck & Co Inc in December 2000. This competitive, reversible inhibitor displays moderate potency (K_i ≈ 130 nM) but has entered phase II clinical trials for the treatment of Type II diabetes. Replacement of the thiazolidide group has been investigated, but no other substituents give improved potency within this series of compounds [22]. More potent compounds have been developed which take advantage of the required interaction with the serine hydroxyl group of the enzyme. Dipeptide diphenylphosphonates (eg, 2, Georgia Institute of Technology; Figure 1) are potent inhibitors but are irreversible [23]. Some of the most potent DPP-IV inhibitors reported are dipeptide boronic acid derivatives (eg, 3, Boehringer Ingelheim Pharmaceuticals Inc; Figure 1) (IC_{50} = 15 nM). These prolineboronic acid (boroPro) derivatives are reversible, slow-binding inhibitors; however, they display poor stability in weakly basic buffer due to an intramolecular cyclization between the N-terminal amino group and the boronic acid [24]. The

Figure 1. Dipeptide-like inhibitors.

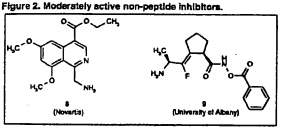


cyclized compounds are inactive *in vitro* but are active *in vivo* because the cyclization is reversible in acidic conditions. The boroPro derivatives show poor selectivity over other proteases such as DPP-II. Similar stability problems are encountered when other electrophilic groups such as aldehydes or ketones are attached to the P_1 residue. Nitriles, however, appear not to suffer the same stability problems. FE-999011 (4, Ferring BV; Figure 1) is a member of a series of 2-cyanopyrrolidide derivatives claimed by Ferring in WO-09515309 [103••] that display potency (K_i = 2.2 nM) comparable to the boroPro derivatives but with much improved stability, $t_{\frac{1}{2}}$ > 48 h at pH 7.4 [25]. The cyano group is sufficiently electrophilic to interact with the serine hydroxyl, but does not cause stability problems. The corresponding thiazolidide derivatives show improved activity (K_i = 0.41 nM) but less stability ($t_{\frac{1}{2}}$ = 27 h at pH 7.4) [26]. These inhibitors are slow binding, reversible inhibitors. However, within this series, compounds lacking the cyano group retain some inhibitory activity. A wide range of side chains on the α-carbon of the P_1 residue is well tolerated. Closely related 2-cyanopyrrolidide derivatives display good selectivity over DPP-II [27]. FE-999011 suppresses plasma DPP-IV activity for 12 h after a single oral dose [28•]. Chronic treatment with this compound in the Zucker diabetic fatty (ZDF) rat postponed the development of diabetes with a twice-daily dose delaying the onset of hyperglycemia by 19 days. These results suggest that this compound may be useful in preventing the progression from impaired glucose tolerance to Type II diabetes.

A series of *N*-glycyl-2-cyanopyrrolidides have been reported by Novartis AG in its 1998 PCT application, WO-09819998 [104••] in which the N-terminal amino group is substituted. NVP-DPP-728 (5; Figure 1) has a comparable *in vitro* potency (K_i = 11 nM) to the compounds bearing a side chain on the α-carbon atom and shows good stability ($t_{\frac{1}{2}}$ = 72 h at pH 7.4). The corresponding thiazolidide analogs, claimed in Novartis's US-06110949, again show improved potency [105]. In this series the cyano group is essential for activity. A kinetic study [29] determined that these compounds are reversible inhibitors of DPP-IV. NVP-DPP-728 shows good activity in models of impaired glucose tolerance, improves insulin secretion and glucose tolerance in Zucker fatty rats [30•] and is currently in phase II clinical trials for the treatment of Type II diabetes. Two other peptidic inhibitors of DPP-IV have been reported. The tryptophan derivative TSL-225 (6, Tanabe Seiyaku Co Ltd; Figure 1), derived from a natural product lead, shows only moderate activity (IC_{50} = 5.7 μM) [31]. A cyclic peptide (7, Université de Versailles; Figure 1) has been reported as an irreversible inhibitor with good potency (IC_{50} = 3 nM); inhibition lasts for several hours [32]. Several non-peptidic inhibitors of DPP-IV have been reported but these display only modest activity. Novartis AG has investigated a series of isoquinoline derivatives, the 4-ethoxycarbonyl analog (8; Figure 2) being the most active (IC_{50} = 0.32 μM) [33]. The University of Albany's fluoroolefin derivative 9 (Figure 2) is an irreversible inhibitor with moderate potency (K_i = 188 nM) and good stability ($t_{\frac{1}{2}}$ = 103 h at pH 7.6). It is reported that the fluoroolefin mimics an amide bond [34].

This review will cover patents and patent applications published in the period January 2001 to May 2002. The initial interest in DPP-IV inhibitors and their possible role in immunology has not been pursued due to a lack of positive results. There has been renewed interest in DPP-IV inhibitors since the possibility of their use in the treatment of Type II diabetes was established. This has led to an increase in the number of patent applications for novel compounds and indications for their use. This interest is understandable since diabetes, in particular Type II diabetes, affects a large and still growing patient population that cannot be adequately treated with existing therapies. In the recently published patent literature there

Figure 2. Moderately active non-peptide inhibitors.



8 (Novartis) 9 (University of Albany)

is a strong emphasis on the treatment of Type II diabetes and most of the supporting data reflect this. DPP-IV inhibitors fit into two main categories, namely dipeptide-like and non-peptidic inhibitors. Prodrugs of the dipeptide-like compounds are considered to constitute a third category. The majority of patent applications cover dipeptide-like compounds closely related to known DPP-IV inhibitors and despite early promise and the availability of large libraries to screen, there has been a lack of applications for novel non-peptide inhibitors.

Dipeptide-like inhibitors

The dipeptide-like inhibitors can be sub-divided into two main classes, those with a side chain on the α-carbon of the P_1 residue, these include compounds which have proline or proline analogs as the P_1 residue and those where the side chain is exclusively on the terminal amino group of the P_2 residue.

Glutamic acid derivatives

Following from the 2-cyanopyrrolidide derivatives previously reported by Ferring [103••] a number of different aminoacyl 2-cyanopyrrolidides have been reported. The nature of the side chain on the α-carbon of the P_1 residue has been further explored and the lipophilic groups of the earlier inhibitors have been replaced by groups bearing other functionality with the hope of achieving extra binding interactions. A recently issued US division, US-06201132 [106], of the original Ferring patent application covers a series of glutamic acid and lysine derivatives of 2-cyanopyrrolidides with extended side chains, as exemplified by the glutamyl derivative 10 (Figure 3) which has K_i = 0.5 nM against the human enzyme. The corresponding descyano compounds are also claimed, although the descyano derivative of 10 is less active (K_i = 150 nM). No *in vivo* activity is given. Probiodrug has also investigated a series of glutamic acid derivatives, which are claimed in WO-00114318 [107]. The application discloses a series of aminoacyl thiazolidides and pyrrolidides, where the side chain of the aminoacyl moiety contains a group that is covalently bound to an oligopeptide of up to 20 amino acid residues. In the examples quoted, the aminoacyl portion is always a glutamic acid residue, eg, compound 11 (Figure 3), which has a K_i = 99 nM against DPP-IV. The binding properties to and the 'transportability' through the peptide transporter PepT1 were measured. Surprisingly, the side chain modifications incorporated into compounds such as 11 had little effect on binding properties that were comparable to Ile-thiazolidide, but transportability through the peptide transporter was drastically reduced.

In a separate experiment, inhibitors with extended side chains were dosed orally to healthy Wistar rats (5 μmol/300 g rat). Inhibition of plasma DPP-IV by these compounds was lower than that observed with Ile-thiazolidide despite their similar K_i values for inhibition of DPP-IV, suggesting that these compounds are significantly less well absorbed from the gut. Compound 11 displayed no systemic effect after oral administration. These results suggest that the compounds can be administered topically with no systemic effects.

α-Carbon side chain derivatives

Ferring has filed two further patent applications that disclose aminoacyl derivatives with extended side chains on the α-carbon of the P_1 residue. The first, WO-00181337 [108] is restricted to a series of lysine and ornithine derivatives with heterocyclic substituents on the ornithine and lysine side chains, exemplified by 12 (Figure 3). The second application, WO-00181304 [109] expands this range of side chains to include heteroatoms, providing a greater range of derivatives exemplified by 13 (Figure 3). The claims in both these applications cover cyano and descyano pyrrolidides and thiazolidides. No biological data are given in either application, except that inhibitory activity against human DPP-IV is observed at concentrations < 300 nM and that the hyperglycemic excursion observed upon glucose challenge in Zucker obese rats was reduced in a dose-dependent manner in animals receiving between 0.1 and 100 mg/kg of compound. Without more specific biological data it is not possible to compare these compounds with other inhibitors.

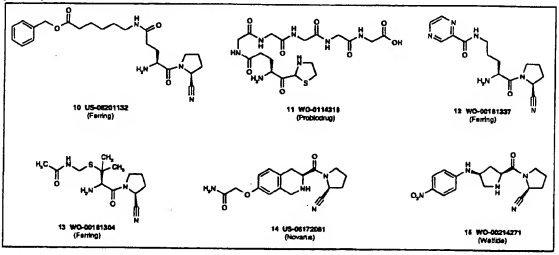
Isoquinolinyl and prolyl derivatives

Novartis has filed US-06172081, which discloses a series of 1-(1,2,3,4-tetrahydro-3-isoquinolinyl)carbonyl-2-pyrrolidine carbonitrile derivatives such as compound 14 (Figure 3) with further substitution on the aromatic ring of the isoquinolinyl moiety [110]. This application is limited to 2-cyano derivatives. The *in vitro* DPP-IV inhibitory activity of the claimed compounds was tested against three different enzyme sources: human colonic carcinoma cell line Caco2, human and rat plasma. Compound 14 displayed IC_{50} values of 16, 5 and 12 nM, respectively. The compounds are claimed for use in treatment of Type II diabetes but no biological data are given. A series of substituted prolyl derivatives such as compound 15 (Figure 3) are disclosed in WO-00214271 filed by Welfide Corp [111]. A wide range of substituents at the 3-position of the proline ring are claimed, giving rise to a potent series of inhibitors with sub-nanomolar potency (eg, compound 15; IC_{50} = 0.18 nM). Substitution at the 3-position is claimed to give improved activity and stability. Compounds lacking the cyano group are also described in this patent application, and some of the thiazolidide derivatives described display nanomolar activity.

Terminal amino group derivatives

The second type of dipeptide-like DPP-IV inhibitors are the *N*-(substituted-glycyl)-2-cyanopyrrolidides first reported by Novartis in WO-09819998 [104••], in which the side chain of the P_1 residue resides exclusively on the N-terminal amino group and not on the α-carbon atom. A further patent application

Figure 3. Pyrrolidide- and thiazolidide-based inhibitors.

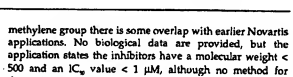


from Novartis, WO-00196295, claims a series of *N*-(substituted-glycyl)-2-cyanopyrrolidides in which the substituent on the terminal amino group is an aliphatic group branched at the α-carbon atom and containing additional functionality as exemplified by the 4-aminocyclohexyl derivative 16 (Figure 4) [112]. This application is limited to 2-cyanopyrrolidide derivatives. The *in vitro* DPP-IV inhibitory activity of the claimed compounds was tested against three different enzyme sources: human colonic carcinoma cell line Caco2, human and rat plasma. Compound 16 displayed IC_{50} values of 5, 10 and 5 nM, respectively. Compound 16 (10 µmol/kg po) administered 10 min prior to glucose challenge gave an 80% inhibition of DPP-IV activity; analysis of blood samples also revealed a 39% decrease in plasma glucose excursion relative to control. The disclosed compounds have similar *in vitro* profiles to those disclosed in earlier applications from Novartis, however, it is difficult to compare the effectiveness *in vivo* with these earlier applications since different parameters were measured. Comparison with other series of inhibitors is not possible since both the *in vitro* and *in vivo* data were obtained using different protocols. This application also claims a combination of the DPP-IV inhibitor along with at least one different antidiabetic agent for treatment of Type II diabetes. In addition to a claim for use of the disclosed compounds in the treatment of Type II diabetes, a method of treatment claim is made for other DPP-IV-mediated conditions such as arthritis, obesity and osteoporosis.

2-Substituted-thiazolidide derivatives

Guilford Pharmaceuticals Inc has filed WO-00134594, which discloses a series of dipeptide-like inhibitors belonging to a family of N-substituted-glycyl derivatives [113]. This application covers a series of 2-substituted-thiazolidide derivatives in which the 2-substituent is selected from tetrazole (eg, compound 17; Figure 4), nitrile, carboxylic or phosphonate groups. A methylene group or an oxygen atom can replace the sulfur of the thiazolidine; in the case of the methylene group there is some overlap with earlier Novartis applications. No biological data are provided, but the application states the inhibitors have a molecular weight < 500 and an IC_{50} value < 1 µM, although no method for determining activity is described. The use of a tetrazole group represents a novel class of dipeptide-like inhibitors, but since the tetrazole group tends to be used as a carboxylic acid mimic it is difficult to see this moiety interacting with the enzyme in the same way as the nitrile. Since there is no biological data available for this series of compounds their effectiveness cannot be compared to other DPP-IV inhibitors.

Figure 4. *N*-(Substituted-glycyl)-2-cyanopyrrolidide derivatives.



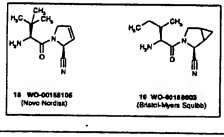
The method of treatment claims include central nervous system disorders such as strokes, tumors, Parkinson's disease, amyotrophic lateral sclerosis and migraine. Surprisingly there is no claim of treatment for diabetes in any form. The claim of treatment for central nervous system disorders is exemplified by data showing that DPP-IV inhibitor c-KPG (compound 6; Figure 1) (IC_{50} = 10 nM) is active in a model employing organotypic spinal motor neurons and threohydroxyaspartate.

Previous work suggests that a pyrrolidine ring, or closely related analog, is the optimum fit into the S1 pocket, hence the previously reported cyano derivatives have all been pyrrolidide or thiazolidide derivatives. Variations on the 5-membered ring have appeared in two recent patent

applications. In one of these, WO-00155105, Novo Nordisk A/S disclosed a series of 2-cyano-3,4-dehydropyrrolidine derivatives that include aminoacyl derivatives bearing side chains on the α-carbon atom of the P_2 residue selected from natural amino acids plus some other bulkier hydrophobic groups (eg, 18; Figure 5) [114]. The claims are extended to include a series of compounds with proline mimics as the P_2 residue. In addition to compounds with a substituent on the α-carbon atom of the P_2 residue, the application claims N-substituted-glycyl derivatives with substituents similar to those claimed by Novartis. This patent application is limited to 2-cyano derivatives. Claims are made for selective and potent inhibitors of DPP-IV, and are also made for a method for treating Type II diabetes. A model of diabetes using ZDF rats is described but no data for the disclosed compounds are included.

In WO-00168603, filed by Bristol-Myers Squibb Co [115], a series of aminoacyl cyclopropyl-fused pyrrolidine-based derivatives containing a nitrile at the 2-position of the pyrrolidine ring are disclosed as DPP-IV inhibitors. The side chain of the aminoacyl group ranges from those present in naturally occurring amino acids, such as the isoleucine analog 19 (Figure 5), to other more elaborate hydrophobic groups, such as adamantyl. β-Amino acid derivatives are also disclosed. No biological data are included although a method for the purification of porcine DPP-IV is described. Since no biological activity is reported it is impossible to say whether the fused cyclopropyl ring provides any benefits over the simpler pyrrolidide derivatives. Claims are made for use in the treatment of diabetes, especially Type II diabetes, both alone and in combination with other antidiabetic agents such as metformin, glyburide, troglitazone, pioglitazone and rosiglitazone.

Figure 5. Other pyrrolidide-based inhibitors.



18 WO-00155105 (Novo Nordisk)

19 WO-00168603 (Bristol-Myers Squibb)

Non-peptide inhibitors

Despite the early identification of non-peptide inhibitors of DPP-IV there have been relatively few recent patent applications for inhibitors of this type.

Fluoroolefin derivatives

Guilford Pharmaceuticals' WO-00134594 [113] which covers dipeptide-like inhibitors, also claims a series of fluoroolefin derivatives of 2-cyanopyrrolidides, such as compound 20 (Figure 6). These compounds are closely related to the fluoroolefin derivatives described previously. The compounds are claimed to be proline mimetics with a molecular weight < 500 and an IC_{50} value < 1 μM, although no method for determining activity is described.

Dihydropurine-2,6-dione derivatives

WO-00202560, filed by Novo Nordisk, claims a series of 3,7-dihydropurine-2,6-dione derivatives as novel DPP-IV inhibitors, as exemplified by compound 21 (Figure 6) [116]. The 1,4-diazepane portion has also been replaced with a piperazine moiety. This application includes examples with a whole range of substituents on this scaffold. No biological data are reported but an assay using porcine DPP-IV and Gly-Pro-pNA as the chromogenic substrate is described. A model using ZDF rats for testing compounds that may be used for treatment and prevention of diabetes is described, but no data are presented. The lack of biological data makes it difficult to compare this novel series of inhibitors with more established types of inhibitors.

Sulphostin

A potent DPP-IV inhibitor, sulphostin (22; Figure 6), has been isolated from the culture broth of *Streptomyces* species MK251-43F3. Zaidan Hojin Biseibutsu Kagaku Kenkyu Kai has filed US-06214340 [117] claiming the compound, its method of production, and reporting an IC_{50} value of 0.008 μg/ml against DPP-IV isolated from rat kidneys. No *in vivo* data are reported and no specific claims for use in the treatment of diabetes are made.

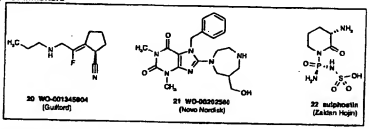
Prodrugs

Two recent patent applications disclosing prodrugs of known DPP-IV inhibitors have been filed. Since some of the more potent inhibitors of DPP-IV have been reported to show poor stability in aqueous buffers with pH > 7.4, the use of prodrugs represents a way of stabilizing the compounds. Additionally, prodrugs of compounds not suffering from stability problems may have improved properties that may enhance their pharmacological activity.

Prodrugs of unstable DPP-IV inhibitors

Probiodrug has filed US-20010020006 disclosing a series of prodrugs of unstable DPP-IV inhibitors [118]. The compound, H-Val-Pro-pyridinium methyl ketone, is an unstable inhibitor of DPP-IV that can undergo intramolecular cyclization between the N-terminal amino group and the ketone, it has a $t_{1/2}$ value of 13.3 min at pH 7.6. Addition of H-Gly-Pro residue to the N-terminus yields compound 23 (Figure 7), which is stable for > 24 h under the same conditions. The active inhibitor is only released in the presence of the enzyme with > 50% of compound 23 detected after incubating for 60 min with DPP-IV. A surprisingly longer duration of inhibition of DPP-IV relative to that observed with the parent inhibitor is observed when the prodrug is incubated with the enzyme and a chromogenic substrate, which is accompanied by a reduced concentration of the prodrug. Although it is claimed that these compounds can be used in the treatment of impaired glucose tolerance and diabetes mellitus, no *in vivo* data are given to support these claims.

Figure 6. Other non-peptide inhibitors



Prodrugs of stable DPP-IV inhibitors

An alternative series of prodrugs not reliant upon degradation by DPP-IV, which appear to display enhanced *in vivo* activity, have been claimed by Ferring in WO-00140180 [119]. The previously reported 1-(2-aminoacyl)-2-cyanopyrrolidide derivatives, which are stable and potent inhibitors, have been further derivatized on the terminal amino group with functional groups including carbamates, alkyloxycarbamates, ehamines and amino acids yielding compounds such as 24 and 25 (Figure 7). These compounds are reported to display no DPP-IV inhibitory activity *in vitro* at 10 µM, indicating that they are at least 1000-fold less potent than the active inhibitors from which they are derived. The prodrugs are metabolized *in vivo* to yield metabolites that are inhibitors of DPP-IV. The *in vivo* activity of these compounds in a glucose tolerance model has been demonstrated in male Zucker fatty rats. Compound 24 (10 mg/kg po) was administered 1 h prior to glucose challenge (1 g/kg). Blood glucose levels were reduced to levels similar to those observed in animals that had been treated with vehicle alone. This reduction in blood glucose levels was comparable to that observed with the same dose of the parent compound. Compounds with other N-terminal groups gave similar effects, but were not always as effective as the reference compound at early time points, indicating that different groups may be metabolized at different rates. The results are consistent with prodrugs that are converted into the parent drug in high yields. In a separate experiment, compound 25 (10 mg/kg po) was administered 12 h prior to glucose challenge. The blood glucose levels for up to 2 h after glucose challenge were reduced to the levels observed in the animals treated with vehicle alone. The prodrug enables significant antihyperglycemic activity to be maintained for up to 12 h.

Indications for DPP-IV inhibitors

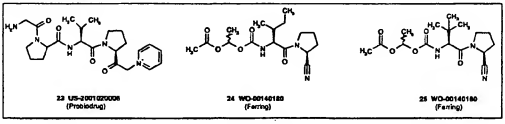
The prime indication in the majority of the recently published patent applications is treatment of diabetes. The first patent to claim the use of DPP-IV inhibitors in the treatment of diabetes mellitus, US-06303661 originated from Probiodrug [120••]. This patent has been granted in the US and the same claims have also been granted in Europe. The patent claims a novel method for the reduction of elevated blood glucose levels by inhibition of DPP-IV-like activity. The claims are exemplified with the use of Ile-thiazolidide. Results include the inhibition of degradation of GLP-1 in rats with a 0.1 mg/kg intravenous dose of Ile-thiazolidide. The modulation of the insulin response and the reduction of blood glucose levels in rats were monitored after administration. Circulating glucose and insulin responses to intraduodenal administration of glucose were also monitored. A dose-dependant reduction in glucose levels was observed on treatment with the inhibitor relative to untreated control; this effect was reversed upon termination of infusion.

In addition to utilizing DPP-IV inhibitors in the treatment of diabetes, two patent applications have been filed for their use in the prevention of diabetes. Novo Nordisk's WO-00162266 claims a method for preventing β-cell degeneration by administration of a DPP-IV inhibitor [121••]. The claims are also extended to cover the use of DPP-IV inhibitors in delaying the progression of impaired fasting glucose to Type II diabetes and the progression of non-insulin-demanding Type II diabetes to insulin-demanding Type II diabetes. Further claims are also made for the use of DPP-IV inhibitors in increasing the size of β-cells. The claims are exemplified by the use of the weakly-active DPP-IV inhibitor Val-pyrrolidide, which was administered orally for 4 days at a dose of 20 mg/kg to male Sprague-Dawley rats on which a 60% pancreatectomy had been performed. In 5/6 animals treated with the inhibitor, insulin was expressed in islets located in the regenerating pancreatic tissue, indicating that Val-pyrrolidide accelerates the differentiation process in regenerating tissue leading to faster formation of new β-cells. The results are in agreement with the decrease in glucose excursion seen in an oral glucose tolerance test.

Probiodrug has disclosed a method of treating mammals to increase the relative insulin producing performance of endogenous pancreatic β-cells in WO-00172290 [122]. This application claims that the oral administration of a DPP-IV inhibitor causes the active form of GLP-1 to be preserved longer under physiological conditions and is exemplified in healthy male subjects in which the active GLP-1 concentration was increased by approximately 300 to 400% following oral administration of Ile-thiazolidide 10 min prior to glucose challenge. In the same study, insulin levels were decreased at doses of 120 to 240 mg/kg, and glucose concentrations showed a significantly lower increase at doses of 15 to 240 mg/kg. The extended presence of GLP-1 facilitates the differentiation and regeneration of β-cells that are in need of repair and that in turn can contribute to the correction and maintenance of normal glycemic levels. A further study in Zucker obese rats involved once a day administration of Ile-thiazolidide (21.61 mg/kg) over a 21-day period.

In addition to the sole use of DPP-IV inhibitors for treatment of diabetes, the combination of DPP-IV

Figure 7. Selected prodrugs.



inhibitors with other antidiabetic agents has been reported as advantageous. Novartis has filed WO-00152825 relating to the combination of a DPP-IV inhibitor with at least one other antidiabetic agent for prevention, delay in progression or treatment of conditions mediated by DPP-IV, in particular Type II diabetes and impaired glucose tolerance [123]. The preferred DPP-IV inhibitors are those claimed in Novartis' earlier patent applications, while the partner compound is selected from a wide range of marketed antidiabetic agents including nateglinide, repaglinide, metformin, acarbose and troglitazone. The dosing regime is dependent upon the partner drug. No biological data are given, but claims are made that in established test models of diabetes the combination of a DPP-IV inhibitor with at least one other compound results in a more effective prevention for treatment of conditions mediated by DPP-IV.

Smithkline Beecham plc (now GlaxoSmithKline plc) has made similar claims using Ile-thiazolidide as the partner DPP-IV inhibitor in WO-00197808 [124]. This PCT application claims a treatment for Type II diabetes by administration of a DPP-IV inhibitor along with another antidiabetic agent. A model using ZDF rats is described in which the rats were dosed orally with Ile-thiazolidide (100 mg/kg) and an antihyperglycemic agent such as a thiazolidinedione insulin sensitizer (5 mg/kg) over a 7-day period. The combination gave a better response with regards to plasma glucose levels compared to administration of the single compound.

Probiodrug's US-06319893 issued on November 20, 2001 claims use of DPP-IV inhibitors in raising blood sugar levels in hypoglycemic mammals [125]. This potential indication is surprising given the well-established role of DPP-IV inhibitors to lower blood sugar levels. It is suggested this apparently contradictory role for DPP-IV inhibitors is possible because they can reduce the degradation of glucagon that is involved in the release of endogenously stored glucose from glycogen. Glucagon has the opposite effect to the incretins. Approximately 2 h after food intake the secretion of incretins is stopped, effectively bringing an end to glucose disposal and after this time glucose levels are maintained by degradation of glycogen. Ile-thiazolidide inhibits the glucagon-degrading activity in plasma with a resultant increase in blood glucose levels relative to saline control in Wistar rats following intravenous administration of glucagon that had been pre-incubated in the plasma of normal rats.

Finally, the use of DPP-IV inhibitors for lowering blood pressure associated with elevated blood glucose levels in mammals has been disclosed in a patent application, US-2002006899, filed by Pospisilik *et al* [126]. The impact of chronic administration of Ile-thiazolidide to ZDF rats on systolic blood pressure is described, effects are observed with an oral dose of 10 mg/kg given twice daily for 100 days.

Conclusion

Despite the availability of large libraries available for screening, the small number of non-peptide-like inhibitors found in the patent literature in the period covered by this review suggests it is more difficult to find new classes of potent inhibitors as opposed to those developed on the basis of the substrate requirements of the enzyme. However, the first generation of DPP-IV inhibitors have not only yielded important research tools but also provided potential drug candidates. A wide range of low molecular weight dipeptide inhibitors have been reported that display good potency and also possess the drug-like qualities required by present day drug discovery programs. These inhibitors also display good activity in *in vivo* models with various routes of administration, including topical and oral. The focus of attention has been for use in the treatment of Type II diabetes, and early results look promising. In addition, there is early evidence to suggest they may be useful in the prevention of diabetes.

Since DPP-IV is located throughout the body and has many different roles it is likely that new therapeutic indications for DPP-IV inhibitors will be identified. Because the current inhibitors display a wide range of physicochemical properties and can be administered by various routes there is a large scope for future developments with regards localized administration. The same properties may also be utilized in designing long- and short-acting drugs. New classes of inhibitors are likely to be forthcoming and will increase the therapeutic options currently available.

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